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Combining ballast water treatment and ballast water exchange: Reducing colonization pressure and propagule pressure of phytoplankton organisms

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Species richness and species abundance (colonization pressure and propagule pressure, respectively) are commonly used to characterize invasion risk for ballast-water-mediated introductions, which can be high if either parameter is high. For practical reasons, the adopted IMO-D2 standard for organisms in discharged ballast water only considers total abundance of biological indicators, without consideration of species richness or source community. Here we explore the effect of ballast-water source, ballast water exchange, chlorination, or a combination of both (hybrid treatment) on both colonization pressure and propagule pressure for one IMO-D2 size class ($\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$; phytoplankton). A strong reduction of propagule pressure was observed in all experimental trials and taxonomic groups, probably owing to environment conditions inside ballast tanks and treatment effects. However, only the hybrid treatment met the IMO-D2 standard for propagule pressure, while also significantly reducing colonization pressure, from 25 initial groups to 16 final groups. In this treatment, dinoflagellates and diatoms dominated final composition. The impact of different treatments on colonization pressure and propagule pressure was always lower when the vessel was ballasted in a brackish than freshwater port. Our study demonstrates that even treated ballast water compliant with the IMO-D2 standard may still harbor a diverse phytoplankton community, albeit with low individual species abundances. These results might be similar even using a type approved ballast water management systems which usually includes a filter for $> 50 \mu\text{m}$ organisms as a pre-treatment.

Keywords: IMO-D2 standard, phytoplankton, invasive species, vectors of introduction, invasion risk

Introduction

Between three and 10 billion tonnes of ballast water is moved around the world every year by commercial vessels (Gollasch, 2002). Ballast water is recognized as a major vector for the introduction of zooplankton, phytoplankton, fishes, bacteria and viral particles (Carlton, 2010). Large

volumes of ballast water imply high potential propagule pressure (PP) and/or colonization pressure (CP) (number of organisms and species released, respectively), and consequently a high establishment probability for non-indigenous species introduced to new environments (Lockwood et al., 2009; Briski et al., 2014). In order to minimize the transport of organisms in ballast water,

the International Marine Organization (IMO) adopted the International Convention for the Control and Management of Ships' Ballast Water and Sediments (IMO, 2004). This convention calls for, among other things, procedures to exchange at least 95% (by volume) of ballast water (BWE) in open oceanic waters more than 200 miles from the coast and in 1000 m minimum depth. BWE reduces the number of species subsequently introduced by physical removal of coastal organisms and, additionally, by killing organisms remaining in ballast tanks owing to osmotic shock (Santagata et al., 2008; Bailey et al., 2011). Although the efficiency of this method depends on the type of BWE (empty-refill or flow through), properties of the source and exchanged waters, and the species involved, it has become a routine practice on commercial vessels over the past twenty years (Goltsch et al., 2007; Gray et al., 2007).

In order to further reduce the probability of ballast-mediated invasions, the IMO adopted the D-2 performance standard (hereafter IMO-D2 standard; see IMO, 2004), which has since been ratified so that it will enter into force in September 2017. The IMO-D2 standard prescribes maximum permissible limits for viable organisms in ballast-water discharge for five biological indicators, including <10 individuals m^{-3} for organisms $\geq 50 \mu m$ in minimum dimension (macroplankton) and <10 individuals mL^{-1} for those between ≥ 50 and $\geq 10 \mu m$ in minimum dimension (phytoplankton), plus three bacteria indicators. Ballast Water Management Systems (BWMS) can be used to meet the discharge standard, and the IMO Convention, through various guidelines, which also provides guidance on the verification testing of BWMS (IMO, 2004).

In parallel, a new approach explored whether a combination of BWE and any of the aforementioned technologies could enhance treatment efficiency through additive or synergistic effects (Lockwood et al., 2009; Briski et al., 2013; Paolucci et al., 2015; see also the US EPA Vessel General Permit). For example, Paolucci et al. (2015) demonstrated under operational conditions that the combination of BWE and chlorination produced greater suppressive effects, and in some cases achieved required numerical limits for size class organisms, than either method by itself.

Heretofore, most attention – including by the IMO – has focused on individual or combined species abundances in ballast water. However, risk of

ballast water is also affected by the number of species introduced, or colonization pressure (Lockwood et al., 2009; Briski et al., 2014). Here we explore the effect of single (BWE, chlorination) or combined treatment of ballast water on both PP and CP using the IMO-D2's phytoplankton size class ($\geq 10 \mu m$ and $< 50 \mu m$). In addition, we examined whether source of ballast water affected variation in CP or PP; we expected that osmotic shock should be more intense for organisms originating in freshwater ports than for those from coastal marine environments (Bailey et al., 2011).

Methods

Samples were collected during experiments conducted on the bulk carrier Federal Venture, provided by Fednav Ltd, during five trips between Canada and Brazil from April 2012 to March 2013 (see details in Paolucci et al., 2015). For the first, third, and fifth trip, the vessel departed from a brackish port, Port Alfred, Quebec, while the second and fourth trips originated in freshwater ports, Trois Rivières and Becancour, Quebec, respectively (Figure 1).

Five of the ten available tanks were dosed with sodium hypochlorite at 20 or 10 ppm during the ballasting process directly to the bottom of each ballast tank, 1 m from the intake pipe's bell mouth



Figure 1. Routes followed during the five trials (solid line) between Canada and Brazil. BWE 1–5 mark the BWE positions for the trips 1–5, respectively, and the solid line circle indicates area where final sampling was conducted (modified with permission from Paolucci et al., 2015).

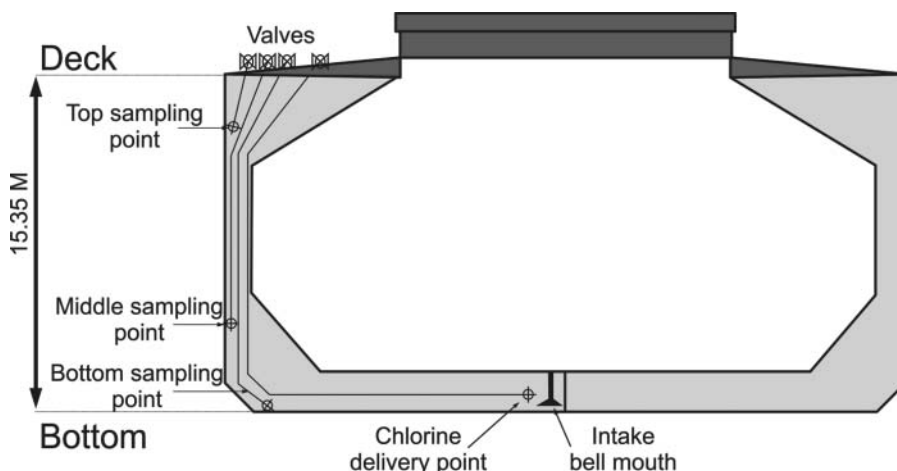


Figure 2. Midship section of the vessel indicating sampling points (top, middle and bottom) and chlorine delivery point on one ballast tank (grey).

(Figure 2). For more details about the procedures involved, see Paolucci et al. (2015). Initial sampling was carried out at port, in parallel to the ballasting and dosing of chlorine, by directly drawing water from a valve located on the starboard ballast pump in the engine room. Each 20 L sample was collected pulling three equals aliquots of unfiltered water taken at different times during the ballasting process, but avoiding the initial and final 20 minutes in order to have a more representative sample (First et al., 2013). Volume was controlled using a Hydrobios flowmeter. After the vessel was at least 200 nm from shore and in at least 1000 m depth, BWE was conducted by the overflow method, according to IMO procedures, on each of three starboard and port tanks.

Final sampling was conducted between three and four days after BWE, collecting representative samples at three different depths according to Murphy (2002) (top, middle and bottom; Figure 2). Ballast water was pumped from each level, and drained forward to the forecabin using a pneumatic peristaltic pump, draining more than 300 L per aliquot. Unfiltered water samples from the different levels of each tank were integrated in 20 L samples (10 final samples in total one in each ballast tank). Salinity was measured *in situ* every time aliquots were collected (triplicate measures) during initial and final sampling using an Orion 130A meter. Other physical and chemical conditions were measured but not reported in this study (Paolucci et al., 2015).

Phytoplankton ($\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$) analysis

Three subsamples of 500 ml were randomly collected from each initial and final sample, (from the 20 L samples) and used for fresh phytoplankton analysis under epifluorescent microscopy (using Fluorescein Diacetate and 5-Chloromethylfluorescein Diacetate), details and results of which are presented in Paolucci et al. (2015). For the present study, all remaining material of each subsample was preserved with ~ 2 ml of Lugol's iodine solution and stored in a cool dark place until analysis at the laboratory never more than two months later. For fixed samples, three replicates subsample were loaded using a micropipette into 1-ml Sedgewick-Rafter counting chambers etched with 1-mm^2 grids. Organisms between 10 and $50 \mu\text{m}$ length were identified at the highest taxonomical level possible and counted at 40–100X magnification under regular light (Leica S8APO). Average total abundance and number of taxa were calculated for each treatment at the initial and final samples. The number of missing groups was recorded as the total number of taxonomic groups that were present at initial samples but absent at final samples.

Statistical analysis

Abundances and richness of all taxonomic groups/samples were log transformed to satisfy

statistical requirements. PP and CP ratios (*r*) were calculated as:

$$r = \log (N_{\text{final}})/(N_{\text{initial}})$$

where *N*_{final} and *N*_{initial} are final and initial densities or richnesses, respectively.

Statistical differences in *r* were analyzed using a block design ANOVA between chlorine and ballast exchange treatments, their interaction, and trial number as a blocking factor. In addition, the effect of chlorine concentration (20 or 10 ppm) or the location of the departure port (Saint Lawrence or Saguenay rivers) was tested by introducing these factors as variables in the ANOVA analysis. We used *post hoc* Bonferroni tests to explore differences in species richness and total abundance ratios of phytoplankton among trips and treatments.

Results

Although initial phytoplankton densities varied widely among trips (Figure 3), significant treatment effects were apparent for both total abundances and richness (Figure 4; ANOVA, *F*_{6, 49} = 6.89, *p* < 0.0012, and *F*_{6, 49} = 4.58, *p* > 0.0094, respectively). On average we noted a strong reduction in total abundance and changes in species' relative importance in all experimental trials, treatments, and taxonomic groups (Table 1). Initial abundance was dominated by diatoms (122.3 ± 191.6 ind ml⁻¹; 63%), followed by dinoflagellates (22.3 ± 17.2 ind ml⁻¹; 11%), green algae

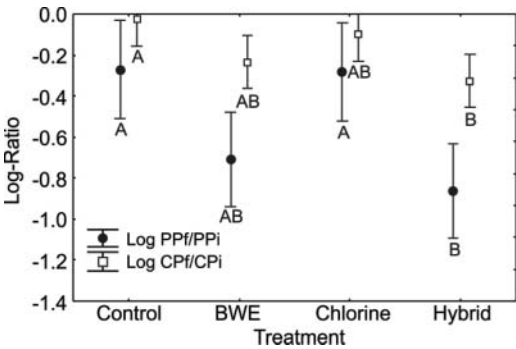


Figure 4. Ratios (±CI) of the final (f) and initial (i) propagule pressure (PP) and colonization pressure (CP) values for controls and three treatments. Letters indicate significant differences between treatments and control tanks. Vertical bars denoted 0.95 confidence intervals.

(8.8 ± 11.5 ind ml⁻¹; 5%) and cyanobacteria (5.7 ± 9.6; 3%). On average, around 18% (36 ind ml⁻¹) of organisms in initial samples could not be identified. A trend of decreasing abundance over time was observed for most phytoplankton groups, except for low abundance taxa such as Desmidiaceae or *Ceratium* sp. in control or BWE tanks, respectively (Table 1).

For all phytoplankton groups (Cyanobacteria, green algae, diatoms and dinoflagellates), the hybrid treatment (BWE plus chlorination) had the lowest final PP and CP, often followed closely by the BWE treatment (Table 1, Figure 3). Mean PP and CP were significantly lower in the hybrid treatment than in other treatments and controls (Bonferroni tests; Figure 4; *p* = 0.0328 and *p* = 0.0196, respectively). Cyanobacteria, green algae and diatoms all exhibited reductions in their relative abundances in both BWE and hybrid treatments, the effect being strongest in green algae in the hybrid treatment (nine times lower). By contrast, in BWE and in hybrid treatments, dinoflagellates increased 2.5–3 fold, respectively, in their final relative abundance (Table 1). BWE and hybrid treatments also had the highest numbers of missing groups, eight and 10, respectively. *Merismopedia* sp., *Rhizosolenia* sp., Desmidiaceae and other colonial and single-spindle green algae were missing in both treatments. Additionally, *Pediastrum* sp. and *Synedra*-like cells were missing following BWE, while *Asterionella* sp., *Scenedesmus* sp., coccoid green algae and filamentous cyanobacteria were missing in the hybrid treatment.

CP and PP in the chlorine treatment were not significantly different from that observed in

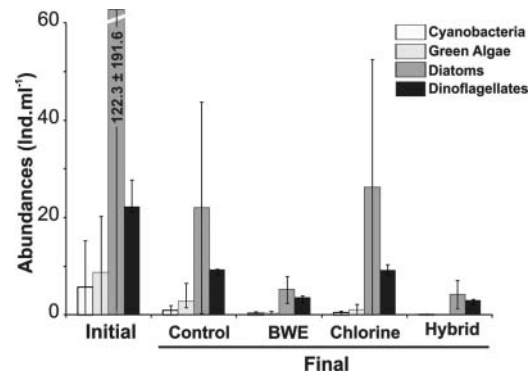


Figure 3. Initial and final abundances of the main taxonomic microplankton groups (cyanobacteria, green algae, diatoms and dinoflagellates) for the control tanks and three treatments (BWE, chlorine and hybrid treatments).

Table 1. Average initial (all tanks) and final (per treatment) phytoplankton taxonomic composition in fixed samples analyzed under light microscopy; percentage composition in the community is given in parentheses. Underlined percentage values display a significantly higher final relative proportion compared to the initial composition. “—” indicates no records for the taxonomic group.

Group/species	Initial Abundance (ind ml ⁻¹) Average all tanks/treatments	Final Abundance (ind ml ⁻¹)			
		Control	BWE	Chlorine	Hybrid
Cyanobacteria (Total)	5.67 (2.91)	0.93 (2.35)	0.33 (2.66)	0.46 (1.14)	0.11 (1.26)
<i>Anabaena</i> like	2.8 (1.44)	0.38 (0.95)	0.02 (0.18)	0.12 (0.31)	0.02 (0.25)
Filamentous no cells	1.53 (0.79)	0.39 (0.98)	0.27 (2.13)	0.21 (0.53)	0.07 (0.76)
Cocoid	0.63 (0.32)	0.07 (0.17)	0.02 (0.18)	0.04 (0.11)	0.02 (0.25)
Other Filamentous-cells	0.61 (0.31)	0.04 (0.11)	0.02 (0.18)	0.08 (0.19)	—
<i>Merismopedia</i>	0.09 (0.05)	0.06 (0.14)	—	—	—
Green Algae (Total)	8.75 (4.48)	2.82 (7.11)	0.38 (3.02)	0.99 (2.47)	0.04 (0.51)
<i>Cocoid</i>	1.99 (1.02)	0.93 (2.35)	0.17 (1.33)	0.24 (0.61)	—
Filamentous cells	1.89 (0.97)	1.19 (3.00)	0.09 (0.71)	0.59 (1.47)	0.02 (0.25)
Other colonial (spindle)	0.28 (0.14)	0.04 (0.11)	—	—	—
<i>Scenedesmus</i> sp.	3.09 (1.59)	0.48 (1.2)	0.12 (0.98)	0.13 (0.33)	—
<i>Pediastrum</i> sp.	0.27 (0.14)	0.07 (0.17)	—	0.02 (0.06)	0.02 (0.25)
Single Spindle	1.2 (0.62)	0.04 (0.11)	—	—	—
Desmidiaceae	0.03 (0.01)	0.07 (0.17)	—	—	—
Diatoms (Total)	122.27 (62.69)	22.01 (55.49)	5.19 (41.44)	26.21 (65.55)	4.18 (47.53)
<i>Diatoma</i>	4.01 (2.06)	1.12 (2.83)	0.30 (2.40)	0.64 (1.61)	0.17 (1.90)
Naviculoid	38.28 (19.63)	10.52 (26.53)	3.57 (28.48)	20.31 (50.79)	3.07 (34.89)
<i>Synedra</i> -like	6.55 (3.36)	0.43 (1.09)	—	0.16 (0.39)	0.02 (0.25)
<i>Asterionella</i>	0.43 (0.22)	0.04 (0.11)	0.02 (0.18)	0.02 (0.06)	—
<i>Melosira</i>	57.84 (29.66)	6.12 (15.43)	0.21 (1.69)	0.21 (0.53)	0.18 (2.02)
<i>Rhizosolenia</i> sp.	—	0.02 (0.05)	—	—	—
<i>Fragilaria</i> oid	2.05 (1.05)	0.29 (0.73)	—	0.53 (1.33)	0.03 (0.38)
Chain (<i>Aulacoseira</i> , <i>S. binderanus</i>)	2.13 (1.09)	0.90 (2.27)	0.02 (0.18)	1.53 (3.83)	0.02 (0.25)

(Continued on next page)

Table 1. Average initial (all tanks) and final (per treatment) phytoplankton taxonomic composition in fixed samples analyzed under light microscopy; percentage composition in the community is given in parentheses. Underlined percentage values display a significantly higher final relative proportion compared to the initial composition. “—” indicates no records for the taxonomic group. (Continued)

Group/species	Initial Abundance (ind ml ⁻¹) Average all tanks/treatments	Final Abundance (ind ml ⁻¹)			
		Control	BWE	Chlorine	Hybrid
Centric non-chain (<i>Cyclotella</i> , <i>Stephanodiscus</i>)	3.41 (1.75)	1.44 (3.64)	0.59 (4.70)	1.46 (3.64)	0.54 (6.19)
Others	7.57 (3.88)	1.13 (2.86)	0.48 (3.82)	1.34 (3.36)	0.14 (1.64)
Dinoflagellates (Total)	22.27 (11.42)	9.31 (23.47)	3.59 (28.66)	9.16 (22.9)	3.03 (34.51)
Dinoflagellates	4.05 (2.08)	0.08 (0.20)	0.28 (2.22)	0.87 (2.17)	0.14 (1.64)
<i>Ceratium</i> sp	0.01 (0.01)	—	0.07 (0.53)	—	—
Dinoflagellate resting cysts	18.21 (9.33)	9.23 (23.28)	3.24 (25.91)	8.29 (20.73)	2.89 (32.87)
Unidentified, grouped by appearance	36.07 (18.49)	4.57 (11.51)	3.01 (24.05)	3.18 (7.95)	1.42 (16.18)
Total (ind.ml⁻¹)	195.04	39.67	12.52	39.99	8.79

control tanks (Table 1, Figure 4; $p > 0.05$; Bonferroni test). In control tanks, the relative importance of cyanobacteria and diatoms decreased slightly over time, while that of green algae and dinoflagellates increased two-fold (Table 1). In the chlorine treatment, Cyanobacteria and green algae relative abundance decreased by half, while that of diatoms remained stable and dinoflagellates two-fold. The only missing group from final control tank samples was *Ceratium* sp., while the chlorine treatment lost six phytoplankton groups (Table 1). In the latter treatment, *Cetarium* sp., *Merismopedia* sp., *Rhizosolenia* sp., Desmidiaceae, and other colonial and single spindle green algae cells were all missing (Table 1).

Significantly lower PP and CP ratios were recorded when the treatments were applied to freshwater versus brackish water, indicating more profound treatment effects (Figure 5; Bonferroni test, $F_{2, 47} = 13.6$, $p < 0.001$ and $F_{2, 47} = 50.4$, $p < 0.001$, respectively). Similarly, Bonferroni contrast test showed strong significant differences in PP of trials 2 and 4 (departing from freshwater ports) as compared to trials 1 and 5 (Figure 6; $F_{4, 30} = 24.0$, p always < 0.001). For CP, the pattern was similar, although not as strong as with PP, with trials 2 and 4 significantly different than trial 5 (Figure 6; $F_{4, 30} = 6.2$, p always < 0.01).

Significant differences in ballast water salinity were recorded between trips at experiment outset, and between those values and final values. Trois Rivières and Becancour (trips two and four) had salinity values close to zero owing to their location on the inner Saint Lawrence River, while ballast water collected from Port Alfred (trips one, three

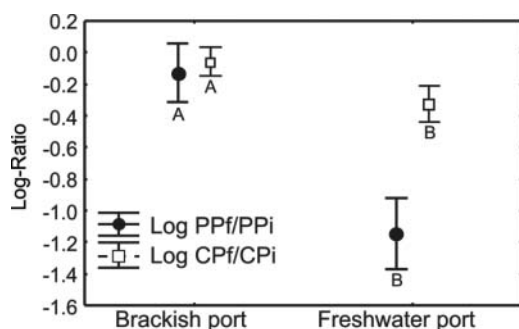


Figure 5. Average (\pm CI) ratios of propagule pressure (PP) and colonization pressure (CP) values for trials starting at freshwater and brackish ports. Letters indicate significant differences. Vertical bars denoted 0.95 confidence intervals. More negative numbers increase greater species abundance or species richness losses.

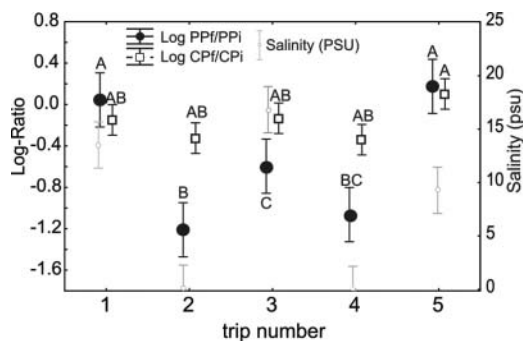


Figure 6. Average (\pm CI) ratios of propagule pressure (PP) and colonization pressure (CP) values (left axis) and salinities values (right axis) for trials starting at freshwater (trips two and four) and brackish ports (trips one, three and five). Letters indicate significant differences among trips. Vertical bars denoted 0.95 confidence intervals.

and five) was significantly higher ($F_{4,15} = 57.3$, $p < 0.001$; Figure 6). Salinity reached 30 psu in all the tanks where BWE treatment was applied, illustrating that volumetric exchange of at least 95% of tank volume was effective. While final salinity values in control and chlorine treatments for all trials were similar to those recorded during initial sampling, final values were, as expected, significantly higher in treatments that involved open ocean BWE for all trials ($F_{3,32} = 8.37$, $p < 0.001$).

Discussion

Management of ballast water, despite its limitations, is a valuable tool to prevent introduction of non-indigenous species (Gray et al., 2007; Bailey et al., 2011). The forthcoming IMO D-2 standard will impose numerical limits for viable organisms in ballast discharge and should reduce further the probability of additional species introductions (Bailey, 2015). However, variation in permissible limits for different sized organisms may provide differential protection against invasions (Gollasch et al., 2007; Hallegraeff, 2015). Our results, as well as those from previous studies (Briski et al., 2013; Paolucci et al., 2015), support the application of a combined strategy of mid-ocean exchange and ballast water treatment as the best option to not only meet the IMO D-2 standard, but also to reduce CP. The analysis of material in fixed samples, which may also include non-viable organisms, allowed us to demonstrate – in a conservative way – that the application of BWE

followed by hypochlorite treatment resulted in significantly lower richness species (CP).

The light microscopy analysis allowed us to identify different taxonomic groups, and to study changes in CP between treatments. Regarding CP, our results demonstrated that the hybrid treatment was the best option to significantly reduce the number of species and, hence, risk of invasion success (Blackburn et al., 2011; Céliavillac et al., 2013; Briski et al., 2014). The other treatments – BWE and chlorine – did not significantly reduce CP, having effects only on PP, or meet the IMO-D2 standard. This aspect has not received as much attention in invasion ecology – particularly that pertaining to ballast water – and was not included in the forthcoming IMO-D2 standard (Gollasch et al., 2007; Briski et al., 2014). We remain troubled by the prospect of treated water in compliance with the IMO-D2 standard that nevertheless continues to constitute an invasion risk owing to little or no reduction in CP (also see Briski et al., 2014; Chan et al., 2014).

In addition, we observed a relative increase in dinoflagellates during the trips, regardless of the applied treatment (Figure 3). Consequently, these algae became the dominant group, along with diatoms, in the final samples. Increases in dinoflagellates following BWE have been reported previously, and seemingly poses a special risk considering some taxa within this group are hazardous to mariculture and human health (Hallegraeff, 2015). Assemblages dominated by diatoms and dinoflagellates were typically reported in ballast tanks that did not received any treatment, after BWE, or other treatments (Dickman and Zhang, 1999; Céliavillac et al., 2013). Despite of the good results obtained by laboratory- and bench-scale studies to reduce algae concentrations (Gregg and Hallegraeff, 2007), the formation of cysts and resting spores by dinoflagellates and diatoms, respectively, may increase their resistance to chemical and physical treatments or ballast water conditions (Galil and Hülsmann, 1997; Gregg and Hallegraeff, 2007). These issues, and the lack of attention to dinoflagellates by the IMO's adopted standard, require further consideration about application of new technologies to control this group, and possibly additional maximum permissible limits for this group or precautions in order to prevent the introduction of nuisance species.

The lower reduction in both PP and CP that we observed when the vessel transported brackish

than freshwater ballast water, regardless of the applied treatment, highlights that water origin should be considered in risk analysis. In the light of these results, we suggest that the application of a hybrid strategy provides the strongest protection against future invasions associated with treated ballast water. The effect of a hybrid strategy seems greatest when source water is freshwater ballast, though a benefit is also evident with brackish source water. It is worth noting that although our results highlight the importance of the analysis per species or taxonomic groups in assessing invasion risk, while the soon-to-be-implemented IMO-D2 standards consider only abundance of biological indicator groups. We propose that abundance-based standards provide incomplete assessments of real risk when managing complex and species-rich assemblages, such as those associated with ballast water.

Conclusions

Our study provides support for the concept of dual treatment of ballast water through application of a hybrid strategy (exchange plus chlorination), as this treatment provided the greatest reduction in plankton abundances, and hence the greatest environmental protection. The utility of the hybrid strategy seems greatest when the source water in ballast tanks is fresh water, though the effect is also evident with brackish water sources. In addition, our study addressed taxonomic abundance of plankton, whereas IMO-D2 standards consider only abundance of viable organisms. Abundance-based standards may provide an incomplete assessment of risk.

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